

**ASSOCIATIONS BETWEEN URINARY CONCENTRATIONS OF NON-
PERSISTENT PESTICIDE EXPOSURE, POLYCYCLIC AROMATIC
HYDROCARBONS AND CARDIOMETABOLIC HEALTH**

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A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

GRADUATE PROGRAM IN KINESIOLOGY AND HEALTH SCIENCE

YORK UNIVERSITY

TORONTO, ONTARIO

AUGUST 2014

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ABSTRACT

Recent literature has established environmental pollutants to be associated with health risk. However, it remains unclear whether the less studied organophosphate (OP) pesticides and polycyclic aromatic hydrocarbons (PAH) influence cardiometabolic health independent of BMI. The first study in this thesis used 2,227 participants from the National Health and Nutrition Examination Survey (NHANES). It was demonstrated that most OPs are associated with a detrimental cardiometabolic health outcome with higher BMIs amplifying health risk. However one metabolite (dimethylphosphate) was associated with advantageous health outcomes. In the second study, 6,159 NHANES participants were used to demonstrate that high levels of PAH are positively and negatively associated with obesity. PAH was also associated with a greater risk of metabolic syndrome, dyslipidemia, hypertension, and type 2 diabetes, independent of BMI. Thus, OP and PAH influence obesity-related health risk, however, more research is needed to further elucidate the mechanistic pathways associated with OP, PAH and health.

ACKNOWLEDGEMENTS

Tell me and I forget, teach me and I may remember, involve me and I learn –

Benjamin Franklin. Thank you to my committee members, Dr. Michael Rotondi and Dr. Chris Arden for your insight, helpful suggestions, comments, and edits of both studies. Thank you to my teacher and supervisor, Dr. Jennifer Kuk, for instilling in me a sense of curiosity and passion for research. Thank you for being patient with me as we went through draft after draft of work, and thank you for believing in me a heck of a lot more than I believed in myself.

What is a friend? A single soul dwelling in two bodies. – Aristotle. Thank you Karissa for showing me the ropes and giving me hope. Thank you Ruth for all of your help and encouragement. Thank you Thiru for being a beacon of advice and for your honesty. Thank you Mike and Dishay for lightening the mood and for always being positive. Finally, thank you Sarah, for whom this experience would not have been nearly as memorable. I cherish the late nights and weekends we spent together and all the outrageous and silly situations we managed to find ourselves in. I can't imagine having gone through this experience without you.

In family life, love is the oil that eases friction, the cement that binds closer together, and the music that brings harmony - Friedrich Nietzsche. Thank you to my parents who never falter in their love for me, who have made sacrifices for me, and whose aim is to see me happy. Thank you to my brother, Shervin, for pushing me to take risks and make change, for inspiring me to be proactive, and for always being supportive of me.

What lies behind us and what lies before us are tiny matters compared to what lies within us – Ralph Waldo Emerson

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LIST OF ABBREVIATIONS

BMI	Body Mass Index
CDC	Center for Disease Control and Prevention
CRP	C-reactive protein
CHD	Coronary heart disease
CVD	Cardiovascular disease
DAP	Dialkyl phosphate
DBP	Diastolic blood pressure
DEDTP	Diethyldithiophosphate
DEP	Diethylphosphate
DETP	Diethylthiophosphate
DMDTP	Dimethyldithiophosphate
DMP	Dimethylphosphate
DMTP	Dimethylthiophosphate
GLU	Plasma glucose
HbA1c	Glycohemoglobin
HDL	High-density lipoprotein cholesterol
HOMA-IR	Homeostatic assessment of insulin resistance
LDL	Low-density lipoprotein cholesterol
LSM	Least squares means
MEC	Mobile examination center

MetS	Metabolic syndrome
NHANES	National Health and Nutrition Examination Survey
NWMP	National Waste Minimization Program
OP	Organophosphate
PAH	Polycyclic aromatic hydrocarbons
PIR	Poverty income ratio
PR	Prevalence risk
SBP	Systolic blood pressure
T2D	Type 2 diabetes
WC	Waist circumference

CHAPTER 1.0 GENERAL INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in the United States (1) and the world (2). The economic burden of CVD is astronomical, costing the U.S an estimated 500 billion dollars in direct and indirect costs in 2010 (3). Health conditions including type 2 diabetes (T2D), hypertension, dyslipidemia, metabolic syndrome (MetS), inflammation, and obesity are major risk factors for CVD (4). Due to its high economic burden and its relation to disease and mortality, researchers have attempted to identify the risk factors associated with CVD. The overwhelming majority of studies have focused on demonstrating the significant influence health behaviours such as diet and physical activity has on CVD (5,6), however, other possible factors contributing to cardiometabolic health has gone largely unnoticed.

The environment is one aspect of cardiovascular health that has received less attention. With the increased frequency and quantity of pesticide use, the likelihood of exposure to such pollutants by the general public is high. Of the few studies examining the effects of pesticides and environmental pollutants on health, the majority report detrimental health effects associated with high exposure. For example, Brook et al. report on the negative impact of air pollution on CVD in a comprehensive literature review (7). Persistent organic pesticides (pesticides that remain in the environment for long periods of time) have also been found to increase health risk for T2D, obesity (8) and even CVD (9).

However, non-persistent pesticides such as organophosphates (OPs) and certain environmental pollutants such polycyclic aromatic hydrocarbons (PAH) have not been

examined to the extent of some of their counterparts. This thesis will investigate: 1) the influence of OP pesticide on the relationship between body mass index (BMI) and cardiometabolic health risk; 2) the association between obesity and PAH in the general population and whether PAH influence the relationship between BMI and T2D, dyslipidemia, hypertension, and MetS.

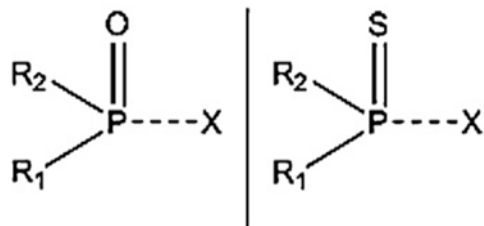
CHAPTER 2.0 REVIEW OF RELATED LITERATURE

Introduction on Organophosphate Pesticide

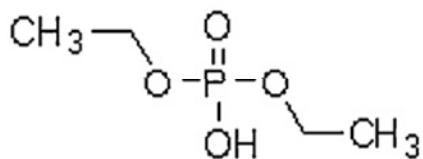
The OP class of insecticides gained popularity after the outlawing of organochlorine based pesticides in 1972 (10). OPs are primarily utilized in agricultural settings but are also available to the public for residential use. The wide array of commercial and residential access to this pesticide makes exposure to the general public unavoidable (11).

OPs are highly effective and, unlike the persistent organochlorine based pesticides such as DDT, have the ability to quickly degrade through hydrolysis when they come into contact with sunlight, soil or air (12). Although easily degraded, OPs maintain high acute affectivity which is due to the pesticides immediate targeting of the central nervous system (12). In specific, the OP pesticide inhibits the production of acetylcholinesterase, an enzyme needed to break down acetylcholine, causing the accumulation of poisonous levels of acetylcholine in the synaptic cleft (11). This compromises the neurons ability to communicate with neighboring neurons, leading to impaired motor functions and ultimately resulting to death in insects (11). Fortunately, humans and large mammals have the ability to process and expel the pesticide from their system within hours or days after exposure (12). The unique characteristics of OPs make them a prime candidate for use around the world, increasing their likelihood of influence on human health.

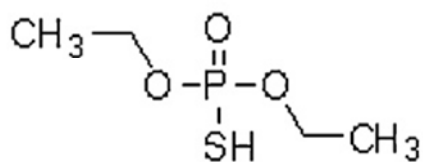
Organophosphorus Pesticides



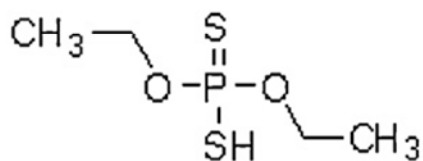
Dimethylphosphates



Dimethylphosphate (DMP)

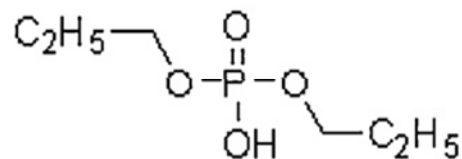


Dimethylthiophosphate (DMTP)

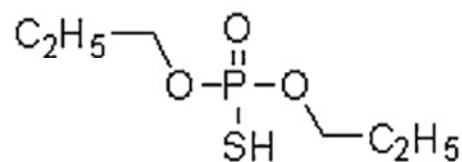


Dimethyldithiophosphate (DMDTP)

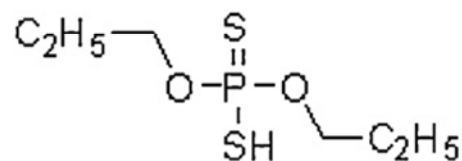
Diethylphosphates



Diethylphosphate (DEP)



Diethylthiophosphate (DETP)



Diethyldithiophosphate (DEDTP)

Figure 1: Chemical structures of each DAP metabolite (Kavvalakis & Tsatsakis, 2012)

Organophosphate Pesticide

In epidemiological research, there are 6 commonly studied urinary metabolites of OP pesticide exposure known as dialkyl phosphates (DAPs; **Figure 1**). These compounds are primarily made up of esters, amides or thiol derivatives of phosphoric, phosphonic, phosphorothioic, or phosphonothioic acids (13). The DAPs are classified into 3 dimethylphosphate metabolites (dimethylphosphate (DMP), dimethyothiophosphate (DMTP), and dimethyldithiophosphate (DMDTP)) and 3 diethylphosphate metabolites (diethylphosphate (DEP), diethyothiophosphate (DETP), and diethyldithiophosphate (DEDTP)). Biomonitoring of OP pesticides are mostly completed through urinary analysis, but can also be measured in sweat, blood, hair and amniotic fluid. It is important to note that long-term exposure, or an accumulation of exposure, is best measured through hair samples (12).

Organophosphate Pesticide and Glucose Markers

A number of studies have established a relationship between OP and glucose indicators (8,14–21). High levels of OP is believed to damage the pancreas and cause acute pancreatitis by increasing oxidative stress (22). These effects of OP on the pancreas may lead to abnormal blood glucose levels (22). Consequently, a large number of animal studies and a select few human trials have reported OP exposure to be related with increases in plasma glucose (14–16,18,20). For instance, Malekirad et al. compared 187 farmers exposed to OP pesticide with 187 matched controls, and discovered that farmers

exposed to OP had significantly higher fasting blood glucose and glucose tolerance test levels compared to the control group (16).

In addition to the aforementioned study, a study examining over 30,000 licensed pesticide applicators observed OP pesticide exposure to increase the risk for T2D (17). OP exposure is also related to higher insulin levels (18) and insulin resistance (18,19). Although previous literature has established links between OP and diabetes markers, many of these findings were observed in animal subjects. In addition, the limited studies conducted on humans focused primarily on individuals directly exposed to high quantities of the pesticide, such as farmers (16,19) and pesticide applicators (17), with none studying the general public. Further, two out of the three human studies used self-report data to quantify pesticide exposure (16,17), with only one directly measuring pesticide levels (19). Thus, it remains unclear whether lower OP rates measured in the general population would result in similar observations.

Organophosphate Pesticide and Blood Lipids

Previous studies have demonstrated inconsistent findings regarding the relationship between OP and blood lipids. For instance, a study conducted on male albino rats observed a significant decrease in HDL after the administration of different doses of OP pesticide (23). On the other hand, Abdou and El Mazoudy administered OP to 35 female Sprague-Dawley rats and found that exposure resulted in significant increases in HDL (24). There are a number of studies in animals indicating that exposure to OP results in significantly lower (15,23), significantly higher (24–26), and no significant

difference in HDL (27). Considering the important role HDL has on cardiometabolic health, it is essential to clarify these inconsistent findings and to further investigate this relationship in humans.

Unlike HDL, the deleterious effects of OP on triglyceride are well established in animals. For instance, a study on overnight-fasted rats found significant increases in triglyceride 8, 12, and 24 hours post OP pesticide exposure, compared to controls (15). In addition, a number of other animal studies have also found OP pesticide to be associated with increased triglyceride levels (23–25). Mechanisms for such findings are unclear, but it is believed that OP pesticides inhibit the lipase activity of hepatic triglycerides and plasma lipoproteins affecting the physiology of HDL and triglyceride within the body (23).

In summary, there is clearly an association between blood lipids and OPs, however we are unable to extrapolate any conclusions as to whether these results will translate to humans. Therefore we are in need of research specifically investigating these relationships in humans to assess whether low levels of OP found in the general public will match previous literature on animals.

Organophosphate Pesticide and Blood Pressure

OP has been previously reported to increase blood pressure in rodents. One study observing 90-day old male rats found that after the administration of a number of different doses of OP, exposed rats had a 15-20 mmHg increase in blood pressure (28). A second study concluded that rats with pre-existing hypertension exhibit even higher blood

pressure after the administration of OP pesticide (29). The only publication observing this relationship in humans demonstrated that acute ingestion of OP was associated with both prevalent hypo and hypertension, however, no baseline measures were taken to quantify the difference between blood pressure levels before and after OP ingestion (30).

Gordon and Padnos have proposed that this increase in blood pressure may have little to do with cardiac output and more to do with an increase in peripheral resistance. The alterations in blood pressure were suggested to have been the result of the pesticide's ability to directly influence the central and peripheral nervous system pathways (28). Nevertheless, in order for us to deduce any concrete conclusions about the effects of OP on blood pressure, we require more research.

Organophosphate Pesticide and Weight Gain

Lastly, OP is believed to lead to weight gain and obesity (8,19,21,31). In general, rats exposed to low-levels of OP pesticide exhibit an increase in weight and adipose tissue mass compared to controls (31). OP exposure has also been found to be positively associated with increases in waist circumference (WC) in a group of OP exposed farmers (19). It is argued that OP may be disrupting the endocrine system in the body resulting in weight gain (31), however there is little evidence for this theory. Although there is a proven association between obesity and health risk, there is large variation in how obesity-related comorbidities are expressed. It is unclear whether OP-related weight gain is similarly associated with detrimental health outcomes. To our knowledge, no studies have investigated whether OP influences the relationship between health risk and obesity.

Summary of Organophosphate Pesticide

For the most part, it is well established that OP is related to negative health outcomes. However, the overwhelming majority of previous literature has concluded findings based primarily on animal subjects with studies examining human participants being far and few in between. The few examining OP and health in humans have placed focus on individuals with direct high exposure to the pesticide, typically through their occupation, with more common exposure levels observed in the general population being scarcely studied.

Specific Aims:

Aim 1: To investigate whether OP influences the relationship between BMI and cardiometabolic health risk.

Introduction to Polycyclic Aromatic Hydrocarbons

Particulate matter, nitrogen oxides, carbon monoxide, and sulfur dioxide are

amongst the most commonly known

and studied environmental pollutants.

Conversely, one pollutant that has

received less attention is PAH. This

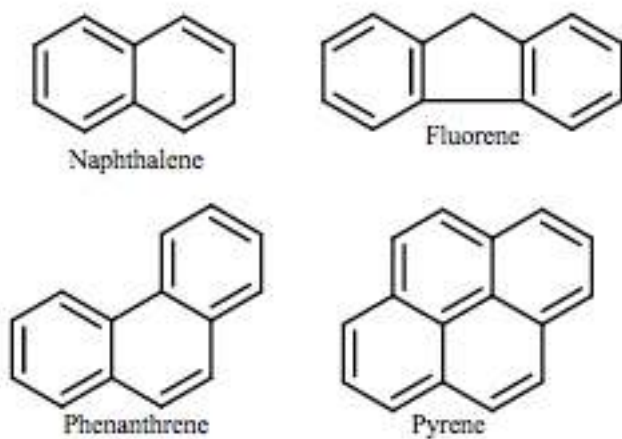
particular environmental pollutant is

formed during the incomplete

combustion of organic substances such

as oil, gas, wood, and coal. PAH are

Figure 2: Chemical structures of 4 PAH



naturally released into the environment through volcanic eruptions and natural forest fires, however humans also contribute to PAH production via activities such as burning of wood and the release of vehicular exhaust into the environment. PAH can be found on charred or grilled meat or in foods such as rice, flour and vegetables grown in spaces where the air or soil is polluted. (32)

Inhalation is the most common method of exposure, however PAH can enter the body through the ingestion of contaminated food or water, or through direct skin contact with polluted soil or other contaminated materials (32). In addition, there is a high positive correlation between certain PAH biomarkers and smoking (33,34), wherein individuals who are exposed to tobacco smoke have shown markedly increased levels of PAH in their urine. Although there are a number of different methods of exposure,

exposure through inhalation is believed to be more readily absorbed by the body, while ingestion shows less absorption (32).

The National Waste Minimization Program (NWMP) reported 31 priority chemicals that they deem toxic and harmful (35). Naphthalene, fluorene, phenanthrene and pyrene are all amongst the priority chemicals listed by NWMP. The basic chemical structures of the 4 PAH mentioned above are illustrated in **Figure 2**. Once exposed, the body processes the chemicals and expels the compounds in its hydroxylated form (36). Urinary metabolites of these chemicals are a reliable indication of exposure to environmental PAH (36–42), which is why NHANES has initiated the biomonitoring of PAH in the urine starting from the 2001 survey year. In specific, NHANES provides participant information on a number of metabolites including 1-naphthalene, 2-naphthalene, 1-fluorene, 2-fluorene, 1-phenanthrene, 2-phenanthrene, 3-phenanthrene, and 1-pyrene.

Polycyclic Aromatic Hydrocarbons and Obesity

Evidence for the relationship between PAH and obesity is lacking with only two studies conducted on youth and none performed on adults. A study by Rundle et al. found that children whose mothers were in the highest tertile of PAH exposure during pregnancy had significantly greater BMI z-scores, and a 79-126% greater risk of obesity compared to children whose mothers were exposed to lower levels (43). Furthermore, using the NHANES datasets, a study examining over 3,000 children found PAH metabolites to be associated with a higher BMI z-score and WC in children aged 6-11

years old (44). Although this relationship has been demonstrated in children, it remains unclear whether PAH is also positively associated with obesity in the adult population.

Polycyclic Aromatic Hydrocarbons and Type 2 Diabetes

In 2030, the prevalence of T2D in adults is projected to rise 69% in developing countries and 20% in developed countries (45) increasing the burden of diabetes and the complications associated with it. Although physical activity, diet, and obesity are well-known risk factors of this chronic disease, recent literature has also established certain persistent organic pollutants to be contributing to the increasing prevalence of T2D (46–48). In addition, PAH increases inflammation in the body (49,50), which is also linked to a greater risk of CVD and T2D (51).

Alshaarawy and colleagues are currently the only researchers to have investigated the association between PAH and T2D. In specific, they observed a positive association between 3 urinary PAH metabolites and T2D, independent of BMI (49). However, with the previous study being the only known study examining this relationship, further research is needed to substantiate these findings.

Polycyclic Aromatic Hydrocarbons and Blood Lipids

Elevated levels of low-density lipoprotein cholesterol (LDL) and serum triglyceride (52,53), and low levels of high-density lipoprotein cholesterol (HDL) (54) are major risk factors for coronary heart disease (CHD) and CVD (4). It is suggested that

over 50% of the U.S adult population has some form of blood lipid abnormality (55).

Environmental pollutants such as PAH may be just one non-traditional risk factor related to abnormal blood lipid levels.

Using the NHANES 1999-2006 survey years, it was demonstrated that certain environmental pollutants were related to abnormal HDL, LDL and triglyceride levels (56). However for the specific PAH pollutant, it was observed that a couple of PAH biomarkers were related to 3% lower levels of HDL, independent of BMI, while no significance was observed for PAH and other blood lipids. Unfortunately, to our knowledge, this was the only study examining PAH and blood lipids. Thus, questions still remain as to whether PAH has a significant association with blood lipids in humans.

Polycyclic Aromatic Hydrocarbons and Cardiac Health

The American Heart Association estimates the prevalence of CVD to increase to 40% and the economic burden to triple by the year 2030 (57). The increased prevalence and financial projections are worrisome, prompting the investigation of possible factors related to the development of CVD and other cardiac-related illnesses. Previous literature has reported PAH to augment the risk of death by ischemic heart disease by 20-40% (58) and to be associated with peripheral arterial disease (59). In addition, 3,283 adults were examined by Xu et al. and it was found that PAH is strongly associated with self-reported CVD (60). The effects of PAH on cardiac health are reflected clearly in the literature, however very limited studies have focused on the conditions known to be major risk factors for cardiac-related illnesses such as hypertension, T2D and dyslipidemia.

Summary of Polycyclic Aromatic Hydrocarbon Literature

It is well established that CVD is strongly related to a number of different health conditions including obesity, T2D, and dyslipidemia. Previous literature has established a clear link between PAH and CVD, however there is very limited literature published on CVDs major risk factors and comorbidities including obesity, T2D and dyslipidemia. Therefore, we are in need of more research to help grow the limited literature currently published in the field.

Specific Aims:

Aim 1: To investigate whether PAH is associated with obesity.

Aim 2: To examine whether PAH influences the relationship between BMI and metabolic syndrome (MetS), T2D, hypertension and dyslipidemia.

CHAPTER 3.0 MANUSCRIPT 1

The Influence of Urinary Concentrations of Organophosphate Metabolites on the Relationship between BMI and Cardiometabolic Health Risk

Organophosphates (OP) are one of the most common types of pesticide used around the world (61). OP pesticides can enter the body through ingestion, inhalation, as well as direct contact with the skin (11,12). Once in the body, the liver processes the pesticide and its metabolites are excreted through the urine (11). The metabolites produced are unique for each pesticide, with the most commonly studied being dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP) and diethyldithiophosphate (DEDTP) (62).

Previous literature has demonstrated OP pesticide exposure to be associated with elevated cardiometabolic risk such as increased triglycerides (15,23–25), HDL (15,23), hyperglycemia (14–17,19,20), and blood pressure (28–30). In addition, positive associations between weight gain and OP pesticides have also been demonstrated (8,19,21,31). However, the majority of studies were performed on animals or individuals with high occupational OP pesticide exposure and it is unclear whether lower levels of OP more commonly observed in the general population are also associated with adverse health effects. Furthermore, as obesity is associated with negative health outcomes, it is also unclear if the influence of OP pesticide on cardiometabolic health risk remains independent of BMI.

Therefore, the main objective of this study is to determine whether OP metabolites modify the relationship between cardiometabolic health risks and BMI in the general U.S population.

RESEARCH DESIGN AND METHODS

National Health and Nutrition Examination Survey (NHANES)

NHANES is a national survey that aims to collect health and diet information from a representative sample of the non-institutionalized U.S. population. NHANES continuous (1999-2013) utilizes a multifaceted probability sampling design that places importance on the oversampling of minority populations. All participants provided written informed consent in agreement with the Public Health Service Act prior to any data collection. Information was acquired from participants through household questionnaires, telephone interviews, and examinations conducted by health care professionals and trained personnel. Data examined in this study was the public use microdata file accessed from the Center for Disease Control and Prevention (CDC) website (63). Information on NHANES survey methods are described in greater detail elsewhere (64,65).

Study Participants and Exclusion Criteria

A total of 51 623 participants were examined during the 1999-2008 survey years. Within this population, NHANES randomly selected a sub-sample of 12 273 survey participants for assessment of OP pesticide exposure using urinary concentrations of six types of dialkyl phosphate (DAP) metabolites. Individuals <20 years of age (n=5452) or pregnant participants (n=417) were excluded from the study resulting in 6467

participants. Individuals with a fasting duration of less than 3 hours or more than 24 hours (n=1231) or those with missing or outlier measurements for high-density lipoprotein cholesterol (HDL; n=296), low-density lipoprotein cholesterol (LDL; n=3626), total cholesterol (n=296), serum triglyceride (n=3467), plasma glucose (n=3443), serum insulin (n=3483), glycohemoglobin (HbA1c; n=243), homeostatic model assessment of insulin resistance (HOMA-IR; n=3488), systolic blood pressure (SBP; n=289), diastolic blood pressure (DBP; n=322), C-reactive protein (CRP; n=277) or body mass index (BMI; n=120) were also excluded. In addition, missing and outlier values for urinary creatinine (n=4), smoking status (n=6), and PIR (n=491) were excluded. One individual with an extreme outlier of 2800 ug/L for DMTP was also excluded leaving a total of 2245 participants remaining participants. Finally, the mean caloric intake was substituted for all individuals with missing caloric intake values (n=278) resulting in 2227 participants available for analysis.

Assessment of Organophosphate Pesticide Exposure

A multi-stage approach was employed for the storage, transportation and measurement of each OP metabolite. Urine specimens were collected and immediately stored at -20°C or lower. Samples were then placed over dry ice and transported to the Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention. Specimen samples that contained less than 0.5 ml of urine were discarded due to the possible occurrence of errors during processing. Urine samples were brought to room temperature and spiked with stable isotope analogues of the specific DAP metabolite being measured, which provided a reliable internal control,

and a lyophilizer was then used to remove excess water from the urine sample. The remaining products were mixed with acetonitrile and diethyl ether, and were chemically derivitized to their individual chloropropyl phosphate esters. Finally, gas chromatography-tandem mass spectrometry was used to assess each individual chloropropyl phosphate esters. These methods are described in greater detail elsewhere (61,66).

Sufficient metabolite concentrations were required for the instruments to accurately detect the OP metabolites, with instrument sensitivity varying depending on survey year. Values below the detection limit were replaced with a value equal to the detection limit divided by the square root of two. For this study, the survey year with the highest detection limit was used as the cut-off value for each metabolite, dichotomizing participants into those above and below metabolite detection limit.

Cardiometabolic Risk Factors

Cardiometabolic risk factors were analyzed using a number of different techniques. In general, trained phlebotomists at mobile examination centers (MEC) obtained blood samples from survey participants. CRP was obtained through latex-enhanced nephelometry of blood specimens (67). The hexokinase method and Roche/Hitachi analyzer were used to evaluate blood plasma glucose (68), while blood HbA1c and insulin levels were obtained by ion exchange high-performance liquid chromatography using the Primus apparatus (69) and the immunoassay method, respectively (70). HOMA-IR was calculated by dividing the product of fasting plasma glucose (mmol/L) and insulin (mU/L) by 22.5. Hitachi analyzers were used to quantify

triglyceride, total cholesterol and HDL levels throughout the survey years while LDL was calculated using the Friedewald equation (71–73). Blood pressure was measured 3 times with some individuals being evaluated 4 times in the event of equipment or technician error. For this study, the mean of all available DBP and SBP measurements was used during analysis. A more detailed explanation of the methods can be found online (63).

Statistical Analysis

Participant characteristics by OP metabolite detection limit status were examined using chi-square tests for categorical variables and t-tests for continuous variables. Continuous variables are presented as means \pm standard error (SE) while the prevalence (N, %) was presented for categorical variables. Multiple regression analysis was performed to assess the association between OP metabolites and BMI. Each regression model was adjusted for potential confounders including age, sex, ethnicity, PIR, smoking status, fasting duration, total caloric intake and urinary creatinine levels to account for urinary dilution level (64). All regression models included an interaction term between BMI and OP metabolites. If no significant interaction was observed, the interaction term was excluded and main effects were examined with adjusted least square means (LSM) \pm SE being computed to illustrate the differences in cardiometabolic health risk by OP metabolite detection status. All data analyses used SAS version 9.3 survey procedures including appropriate weights to adjust for unequal sampling probabilities and to allow representation of the U.S population. A value of $p < 0.05$ was used as the criterion for significance.

RESULTS

Participant characteristics by OP metabolite detection status are presented in **Table 1**. In general, those with detectible OP metabolites were significantly older than those below detection (with the exception of DETP and DEDTP). In addition, BMI was significantly ($P < 0.0001$) associated with all observed cardiometabolic health risk factors after adjustment for confounders.

Table 2 illustrates the association between OP metabolites and cardiometabolic health risks after adjusting for confounders. DBP was observed to be significantly higher for individuals with detectible DMP, DEP or DEDTP ($P < 0.05$) while no significant difference was observed for DMTP, DMDTP, or DETP. Further, individuals with detectible DMTP had significantly lower total cholesterol and LDL than those without detectible levels, while no significant differences were observed for CRP, HOMA-IR, HbA1c, plasma glucose or SBP ($P > 0.05$).

Figure 1 presents the association between HDL and BMI by OP metabolite detection limit status after adjustment for confounders. Individuals with detectible DMTP had significantly higher HDL than those below detectible levels ($P = 0.01$). Contrarily, those with detectible DEDTP had significantly lower HDL levels than those below detection ($P = 0.01$). Individuals with detectible DEP and DETP exhibited lower HDL at the higher BMI range, while a marginal difference was observed between OP metabolite detection statuses at lower BMIs (Interaction effect: $P = 0.034$ and $P = 0.0153$, respectively). No significant interactions or main effects were observed with DMP or DMDTP and HDL ($P > 0.05$).

Figure 2 demonstrates the relationship between triglyceride and BMI by OP metabolite detection limit status after adjusting for confounders. Individuals with detectable DMDTP exhibited significantly higher triglyceride levels than those below detection (Main Effect: $P = 0.05$). In addition, there was a significant interaction effect between DETP and BMI ($P = 0.02$), wherein individuals with detectable DETP had higher triglyceride levels than those below detection at higher BMI ranges and only a minimal difference at lower BMIs. There were no significant interactions or main effects for DMP, DMTP, DEP, or DEDTP metabolites ($P > 0.05$).

BMI was positively associated with insulin levels. Individuals with detectable DEP had higher insulin levels than those below detection at the higher BMI range with smaller differences at a lower BMI. (**Figure 3**; $P = 0.0191$). No significant associations were observed with the other metabolites and insulin ($P > 0.05$).

DISCUSSION

To our knowledge, we are the first to investigate the influence of urinary OP metabolites on the relationship between BMI and cardiometabolic health risk in the general population. We demonstrated that individuals with detectible OP metabolites most commonly have an augmentation of obesity-related cardiometabolic health risk. However, DMTP was associated with a healthier lipid profile for a given BMI. Consequently, these findings suggest that detectible OP metabolite levels in the general population may confer both adverse and advantageous health outcomes, thus highlighting the importance of examining each metabolite individually when studying the effects of OP metabolites on health.

Several studies have demonstrated significant findings in respect to OP pesticide and glucose markers (8,14–21). In general, it is believed that OP pesticide directly effects the functioning of the pancreas leading to an altered glucose profile (22). Consequently, a large number of animal and human studies have observed OP pesticide exposure to be associated with significant increases in plasma glucose (14–16,18,20), insulin (18), insulin resistance (18,19) and incidence of type 2 diabetes (17). Although previous literature has established links between OP pesticide exposure and diabetes biomarkers, we observed that only DMDTP was associated with fasting insulin after adjusting for BMI. The lack of significance between glucose markers and OP metabolites may be partly due to the fact that previous investigation of human exposure to OP pesticides have focused primarily on individuals who are regularly exposed to high quantities of the pesticides through their occupation, such as farmers (16,19) and pesticide applicators

(17). Thus, OP exposure levels seen in the broader population may not be sufficient to elicit the same negative effects.

The association between OP pesticide exposure and HDL remains unclear, with previous studies reporting a number of contradictory findings. Compared to controls, rats exposed to OP pesticides have exhibited significantly lower (15,23), significantly higher (24–26), and no significant (27) effect on HDL. Mechanisms for such findings are unclear, but it is suggested that OP pesticides may influence lipase activity of hepatic triglycerides and plasma lipoproteins (23). We demonstrate that individuals with detectable DEDTP had 0.09 mmol/L lower HDL than those with non-detectable levels for a given BMI ($P = 0.01$). In addition, obese individuals above detection for DEP and DETP had 0.03-0.04 mmol/L lower HDL compared to those below detection. Based on previous observations(54), these findings may translate to 4-15% higher cardiovascular disease (CVD) mortality rates.

Although having detectable levels of certain metabolites was associated with a detrimental health profile, the opposite finding was observed with the DMTP metabolite. In general, individuals with detectable DMTP exhibited significantly higher HDL, lower LDL, and lower total cholesterol than those without detectable levels. Interestingly, these findings are not completely uncommon with previous reports demonstrating that OP pesticide exposure decreases LDL in normolipidemic and hyperlipidemic rats (26) and rabbits (25). As previously discussed, a number of studies have also observed higher HDL levels (24–26) in OP pesticide exposed participants. Although interesting, it remains unclear why this specific metabolite resulted in a more favorable lipid outcome;

however, we believe that these findings place emphasis on the unique physiological effects of each specific OP metabolite. Thus, we are in need of further research examining the possible etiology and physiology of DMTP within the body to advance our understanding of these outcomes.

Previous work on rodents has examined the relationship between OP pesticide exposure and blood lipids with reports that OP pesticide exposed rats exhibit significantly higher triglyceride levels than controls (15,23–25). Consistent with these studies on rats, we demonstrate that individuals with detectible DMDTP have 0.09 mmol/L higher serum triglyceride levels than those below detection, independent of BMI ($P = 0.05$). Further, obese individuals with detectible DEP had 0.17 mmol/L higher triglyceride than those below detection which translates to a 5-13% higher risk of CVD (53). Thus, certain OP metabolites foster unfavorable triglyceride levels, which may modestly augment CVD in the general population, with individuals at a higher BMI range having their risk amplified.

Limited research has examined the effects of OP pesticides on blood pressure, however it has been reported that both low and high doses of OP pesticide is associated with an increase in blood pressure up to 24 hours post exposure in rats (28). In addition, rats with pre-existing hypertension exhibit greater blood pressure increases after the administration of OP pesticide (29). These findings are thought to be the result of OP pesticides directly effecting the central and peripheral nervous system pathways, leading to altered blood pressure outcomes (28). Interestingly, we observed that independent of BMI, individuals with detectible DMP, DEP and DEDTP metabolite levels had a 1-3

mmHg higher DBP ($P < 0.05$) than those below detection which may augment the risk of CHD by 4-13% (74). Therefore, independent of BMI, the association between OP metabolites and blood pressure may amplify risk of adverse cardiac events in the general population.

A few limitations and strengths of this study warrant mention. First, the cross-sectional nature of the present study does not allow us to infer causation. Second, although several previous studies support the use of these six types of dialkyl phosphate metabolites as an indication of OP pesticide exposure (25,75,76), questions on the ability to attribute the source of urinary OP metabolites remain (77,78). Finally, we elected to use more liberal detection limits to reduce the likelihood of misclassifying the exposure, and this study represents one of the few studies examining OP metabolite concentrations and health risk that is more applicable to the general public.

CONCLUSIONS

In summary, we demonstrated that having detectible levels of urinary OP metabolites might be associated with both advantageous and detrimental metabolic outcomes independent of BMI, with obese individuals having an amplification of cardiometabolic health risk. In addition, when studying the effects of OP metabolites on health, it is important to examine the influence of each OP metabolite individually as their effects on health may differ. Consequently, future studies need to examine the physiology of each metabolite, with particular attention on the obese population wherein health risk seems to be amplified.

Table 1A: Participant characteristics for those above and below the detection limit for dimethyl metabolites

	DMP			DMDTP			DMDTP		
	Below (n = 1299)	Above (n = 928)	P Value	Below (n = 740)	Above (n = 1487)	P Value	Below (n = 1636)	Above (n = 591)	P Value
Age (years)	43.5 ± 0.5	45.4 ± 0.6	< 0.01	42.5 ± 0.7	45.3 ± 0.5	< 0.0001	43.7 ± 0.4	45.8 ± 0.7	< 0.01
Sex, n (% Male)	704 (54.2)	452 (48.7)	0.04	386 (52.2)	770 (51.8)	0.25	872 (53.3)	284 (48.1)	0.03
Ethnicity, n (%)			0.78			0.76			0.86
White	651 (51.1)	478 (51.5)		373 (50.4)	756 (50.8)		823 (50.3)	306 (51.8)	
Black	257 (19.8)	177 (19.1)		141 (19.1)	293 (19.7)		318 (19.4)	116 (19.6)	
Other	391 (30.1)	273 (29.4)		226 (30.5)	438 (29.5)		495 (30.3)	169 (28.6)	
PIR, n (% below poverty)	256 (19.7)	151 (16.3)	0.81	146 (19.7)	261 (17.6)	0.35	307 (18.8)	100 (16.9)	0.13
Smoker n (%)			0.10			0.0005			< 0.0001
Never	651 (50.1)	492 (53.0)		372 (50.3)	771 (51.8)		832 (50.9)	311 (52.6)	
Current	335 (25.8)	210 (22.6)		225 (30.4)	320 (21.5)		433 (26.5)	112 (19.0)	
Past	313 (24.1)	226 (24.4)		143 (19.3)	396 (26.6)		371 (22.6)	168 (28.4)	
BMI (kg/m ²)	28.1 ± 0.2	27.9 ± 0.3	0.44	27.6 ± 0.2	28.2 ± 0.2	0.01	28.0 ± 0.2	28.0 ± 0.3	0.90
Creatinine (mg/dL)	127.3 ± 2.3	132.4 ± 3.0	0.12	113.7 ± 3.2	138.3 ± 2.4	< 0.0001	127.0 ± 2.3	136.5 ± 3.4	< 0.01
Total calorie intake (kcal)	2240 ± 33	2188 ± 37	0.17	2178 ± 40	2240 ± 31	0.11	2218 ± 28	2219 ± 42	0.98
Metabolic Variables									
HOMA-IR	2.92 ± 0.09	2.74 ± 0.09	0.10	2.68 ± 0.10	2.93 ± 0.08	0.03	2.84 ± 0.08	2.84 ± 0.12	0.98
Insulin (pmol/L)	67.4 ± 1.9	64.2 ± 1.8	0.15	63.9 ± 2.0	67.2 ± 1.7	0.16	65.8 ± 1.5	66.7 ± 2.8	0.71
HbA1c (%)	5.4 ± 0.1	5.4 ± 0.1	0.86	5.4 ± 0.1	5.5 ± 0.1	0.03	5.4 ± 0.1	5.5 ± 0.1	0.33
Glucose (mmol/L)	5.60 ± 0.04	5.58 ± 0.04	0.68	5.51 ± 0.05	5.63 ± 0.04	0.04	5.59 ± 0.03	5.58 ± 0.06	0.79
HDL (mmol/L)	1.35 ± 0.01	1.37 ± 0.02	0.33	1.33 ± 0.02	1.37 ± 0.02	0.03	1.35 ± 0.01	1.37 ± 0.02	0.39
LDL (mmol/L)	3.11 ± 0.03	3.07 ± 0.03	0.31	3.21 ± 0.04	3.03 ± 0.03	< 0.0001	3.11 ± 0.03	3.06 ± 0.05	0.30
Total Cholesterol (mmol/L)	5.13 ± 0.04	5.11 ± 0.04	0.65	5.21 ± 0.04	5.07 ± 0.03	< 0.01	5.12 ± 0.03	5.13 ± 0.06	0.85
Triglyceride (mmol/L)	1.47 ± 0.03	1.47 ± 0.03	0.56	1.45 ± 0.04	1.47 ± 0.02	0.56	1.44 ± 0.02	1.52 ± 0.04	0.03
CRP (mg/dL)	0.36 ± 0.02	0.39 ± 0.03	0.15	0.36 ± 0.03	0.38 ± 0.01	0.48	0.37 ± 0.02	0.37 ± 0.03	0.85
SBP (mmHg)	120 ± 1	120 ± 1	0.93	119 ± 1	121 ± 1	0.06	120 ± 1	120 ± 1	0.93
DBP (mmHg)	71 ± 1	72 ± 1	< 0.01	72 ± 1	72 ± 1	0.87	71 ± 1	72 ± 1	0.29

DMP = dimethylphosphate, DMDTP = dimethylphosphate, DMDTP = dimethyldithiophosphate

Values are presented as mean ± SE

P values represent the statistical difference between detection status

Table 1B: Participant characteristics for those above and below the detection limit for diethyl metabolites

	DEP			DETP			DEDTP		
	Below (n = 1282)	Above (n = 945)	P Value	Below (n = 1376)	Above (n = 851)	P Value	Below (n = 2109)	Above (n = 118)	P Value
Age (years)	43.6 ± 0.6	45.1 ± 0.6	0.02	44.7 ± 0.4	43.6 ± 0.6	0.11	44.5 ± 0.4	40.6 ± 1.4	< 0.01
Sex <i>n</i> (% Male)	693 (54.1)	463 (49.0)	0.05	701 (50.9)	455 (53.4)	0.10	1098 (52.1)	58 (49.2)	0.30
Ethnicity <i>n</i> (%)			0.55			< 0.01			0.23
White	650 (50.7)	479 (50.7)		742 (53.9)	387 (45.5)		1070 (50.7)	59 (50.0)	
Black	244 (19.0)	190 (20.1)		236 (17.2)	198 (23.3)		409 (19.4)	25 (21.2)	
Other	388 (30.3)	276 (29.2)		398 (28.9)	266 (31.3)		630 (29.9)	34 (28.8)	
PIR <i>n</i> (% below poverty)	239 (18.6)	168 (17.8)	0.75	257 (18.7)	150 (17.6)	0.27	393 (18.6)	14 (11.9)	0.76
Smoker <i>n</i> (%)			0.22			0.17			0.76
Never	672 (52.4)	471 (49.8)		694 (50.4)	449 (52.8)		1086 (51.5)	57 (48.3)	
Current	282 (22.0)	263 (27.8)		357 (25.9)	188 (22.1)		513 (24.3)	32 (27.1)	
Past	328 (25.6)	211 (22.3)		325 (23.6)	214 (25.1)		510 (24.2)	29 (24.6)	
BMI (kg/m ²)	28.0 ± 0.2	27.9 ± 0.2	0.53	28.2 ± 0.2	27.7 ± 0.2	0.06	28.0 ± 0.2	27.6 ± 0.6	0.42
Creatinine (mg/dL)	124.5 ± 2.4	136.1 ± 3.5	0.0004	118.4 ± 2.3	148.5 ± 3.4	< 0.0001	127.9 ± 2.0	156.3 ± 11.2	< 0.0001
Total calorie intake (kcal)	2231 ± 32	2201 ± 35	0.43	2177 ± 29	2287 ± 44	< 0.01	2220 ± 26	2186 ± 71	0.68
Metabolic Variables									
HOMA-IR	2.82 ± 0.09	2.87 ± 0.13	0.63	2.90 ± 0.09	2.74 ± 0.09	0.17	2.85 ± 0.07	2.80 ± 0.21	0.86
Insulin (pmol/L)	65.5 ± 2.0	66.7 ± 2.6	0.60	67.2 ± 1.7	64.1 ± 1.8	0.19	66.0 ± 1.4	67.2 ± 4.7	0.79
HbA1c (%)	5.4 ± 0.1	5.4 ± 0.1	0.55	5.5 ± 0.1	5.4 ± 0.1	0.10	5.4 ± 0.1	5.3 ± 0.1	0.01
Glucose (mmol/L)	5.59 ± 0.05	5.59 ± 0.04	0.95	5.61 ± 0.04	5.56 ± 0.04	0.40	5.60 ± 0.03	5.40 ± 0.07	0.09
HDL (mmol/L)	1.35 ± 0.02	1.37 ± 0.02	0.22	1.36 ± 0.01	1.35 ± 0.02	0.57	1.36 ± 0.01	1.28 ± 0.04	0.02
LDL (mmol/L)	3.08 ± 0.03	3.12 ± 0.04	0.35	3.12 ± 0.03	3.05 ± 0.04	0.08	3.10 ± 0.02	3.05 ± 0.11	0.55
Total Cholesterol (mmol/L)	5.09 ± 0.03	5.17 ± 0.04	0.07	5.15 ± 0.03	5.07 ± 0.04	0.09	5.13 ± 0.03	4.98 ± 0.15	0.11
Triglyceride (mmol/L)	1.44 ± 0.03	1.50 ± 0.03	0.07	1.46 ± 0.02	1.46 ± 0.03	0.97	1.47 ± 0.02	1.43 ± 0.10	0.65
CRP (mg/dL)	0.36 ± 0.01	0.38 ± 0.03	0.37	0.39 ± 0.02	0.34 ± 0.02	0.03	0.37 ± 0.01	0.37 ± 0.08	0.99
SBP (mmHg)	120 ± 1	121 ± 1	0.08	121 ± 1	119 ± 1	0.07	120 ± 1	119 ± 2	0.38
DBP (mmHg)	71 ± 1	72 ± 1	0.0004	72 ± 1	72 ± 1	0.90	71 ± 1	73 ± 1	0.04

DEP = diethylphosphate, DETP = diethyldiphosphate, DEDTP = diethyldithiophosphate

Values are presented as mean ± SE

P values represent the statistical difference between detection status

Table 2: Cardiometabolic risk factors means for individuals above and below OP metabolite detection limit

OP Metabolite	LDL (mmol/L)	Total Cholesterol (mmol/L)	Glucose (mmol/L)	HOMA-IR	HbA1c (%)	CRP (mg/dL)	SBP (mmHg)	DBP (mmHg)
DMP								
Below	3.06 ± 0.08	5.08 ± 0.08	5.78 ± 0.09	3.11 ± 0.18	5.6 ± 0.1	0.41 ± 0.04	122 ± 1	71 ± 1*
Above	3.04 ± 0.09	5.05 ± 0.10	5.74 ± 0.09	2.96 ± 0.15	5.6 ± 0.1	0.45 ± 0.06	122 ± 1	72 ± 1
DMTP								
Below	3.18 ± 0.09*	5.17 ± 0.09*	5.75 ± 0.08	3.01 ± 0.15	5.6 ± 0.1	0.42 ± 0.06	122 ± 1	72 ± 1
Above	2.98 ± 0.08	5.01 ± 0.09	5.77 ± 0.09	3.07 ± 0.17	5.6 ± 0.1	0.43 ± 0.05	122 ± 1	71 ± 1
DMDTP								
Below	3.06 ± 0.08	5.07 ± 0.09	5.78 ± 0.09	3.05 ± 0.16	5.6 ± 0.1	0.43 ± 0.05	122 ± 1	71 ± 1
Above	3.02 ± 0.09	5.06 ± 0.10	5.73 ± 0.10	3.04 ± 0.18	5.6 ± 0.1	0.42 ± 0.05	122 ± 1	72 ± 1
DEP								
Below	3.04 ± 0.08	5.04 ± 0.09	5.77 ± 0.09	3.01 ± 0.17	5.6 ± 0.1	0.42 ± 0.05	121 ± 1	71 ± 1*
Above	3.07 ± 0.08	5.10 ± 0.09	5.76 ± 0.09	3.10 ± 0.19	5.6 ± 0.1	0.43 ± 0.06	122 ± 1	72 ± 1
DETP								
Below	3.07 ± 0.08	5.07 ± 0.09	5.78 ± 0.08	3.10 ± 0.16	5.6 ± 0.1	0.44 ± 0.05	122 ± 1	71 ± 1
Above	3.03 ± 0.09	5.05 ± 0.10	5.74 ± 0.10	2.97 ± 0.18	5.6 ± 0.1	0.41 ± 0.05	121 ± 1	71 ± 1
DEDTP								
Below	3.05 ± 0.08	5.07 ± 0.09	5.77 ± 0.09	3.04 ± 0.16	5.6 ± 0.1	0.43 ± 0.05	122 ± 1	71 ± 1*
Above	3.05 ± 0.12	4.99 ± 0.15	5.67 ± 0.10	3.13 ± 0.21	5.5 ± 0.1	0.42 ± 0.09	123 ± 2	74 ± 1

* Significant difference between detection status ($P < 0.05$)

Values are means ± SE adjusted for BMI, PIR, ethnicity, age, sex, smoking status, urinary creatinine level, fasting duration and total caloric intake

DMP = dimethylphosphate, DMTP = dimethylthiophosphate, DMDTP = dimethyldithiophosphate, DEP = diethylphosphate,

DETP = diethylthiophosphate, DEDTP = diethyldithiophosphate

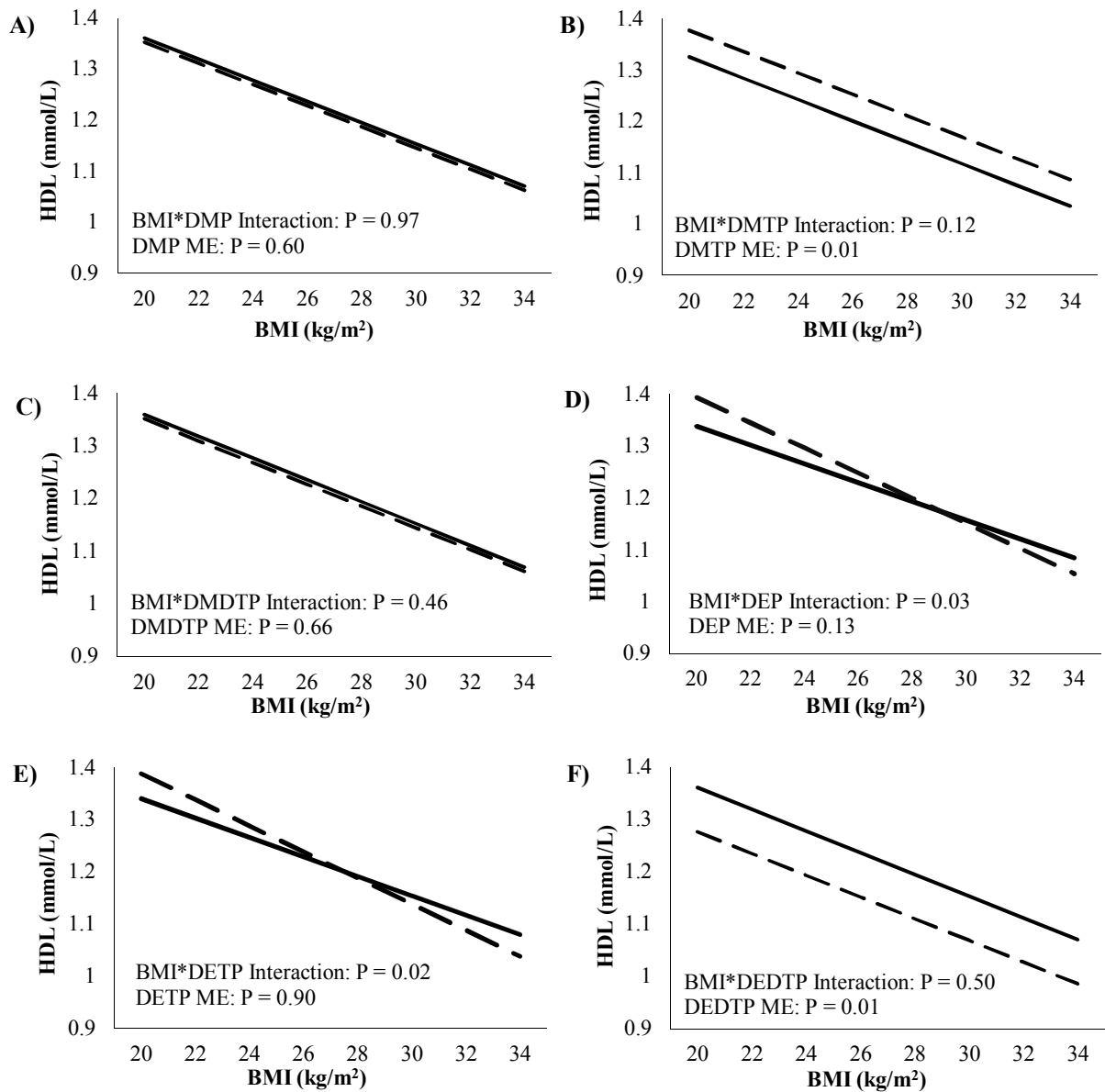


Figure 1 A-F): Relationship between HDL and BMI by DMP (A), DMTP (B), DMDTP (C), DEP (D), DETP (E) and DEDTP (F) detection limit status.

Solid black line represents individuals below the detection limit while the dotted black line represent individuals above the detection limit.

ME = main effect

Models are adjusted for PIR, ethnicity, age, sex, smoking status, urinary creatinine, fasting duration and total calorie intake

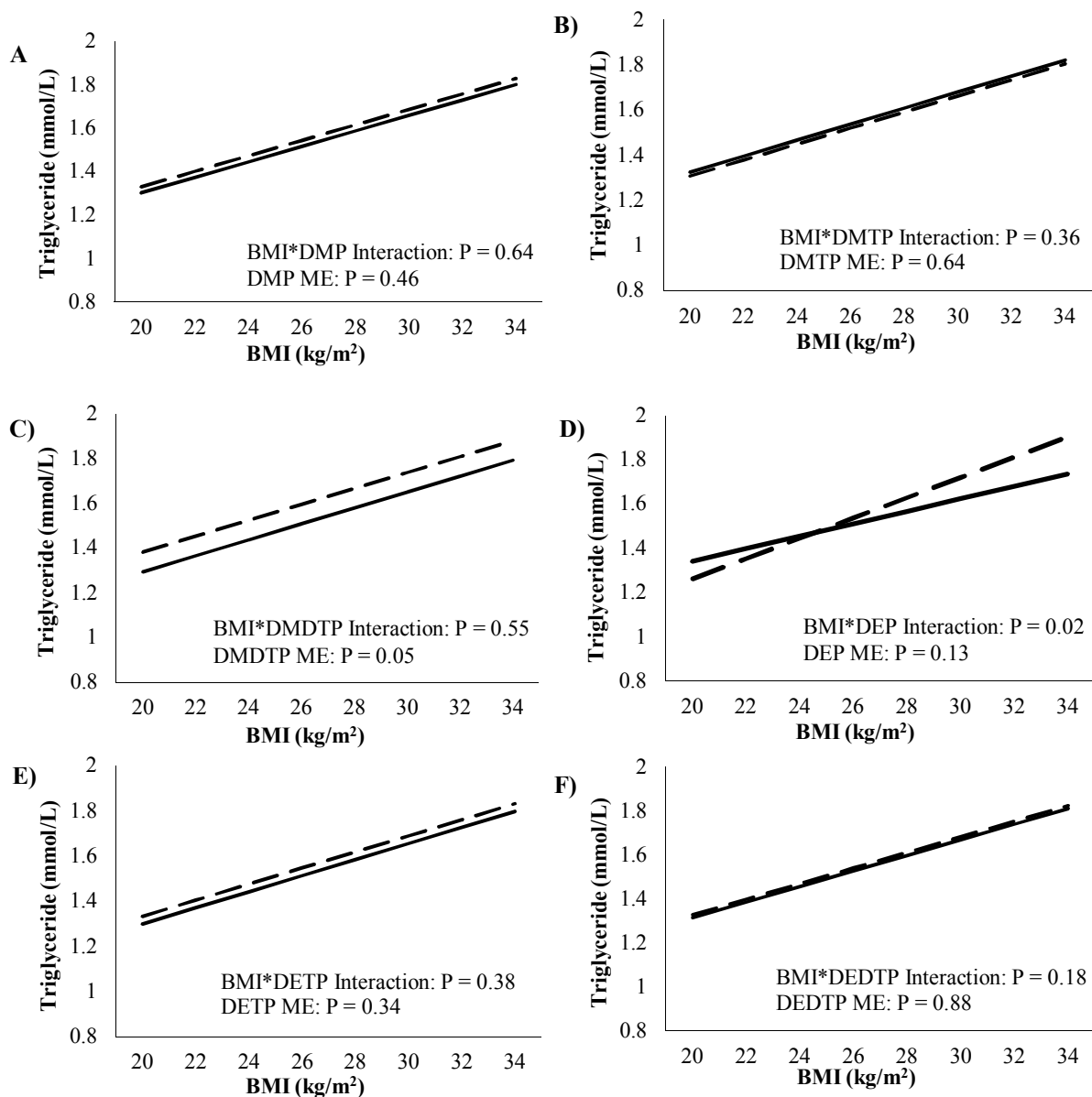


Figure 2 A-F): Relationship between triglyceride and BMI by DMP (A), DMTP (B), DMDTP (C), DEP (D), DETP (E) and DEDTP (F) detection limit status.

Solid black line represents individuals below the detection limit while the dotted black line represent individuals above the detection limit.

Models are adjusted for PIR, ethnicity, age, sex, smoking status, urinary creatinine, fasting duration and total calorie intake

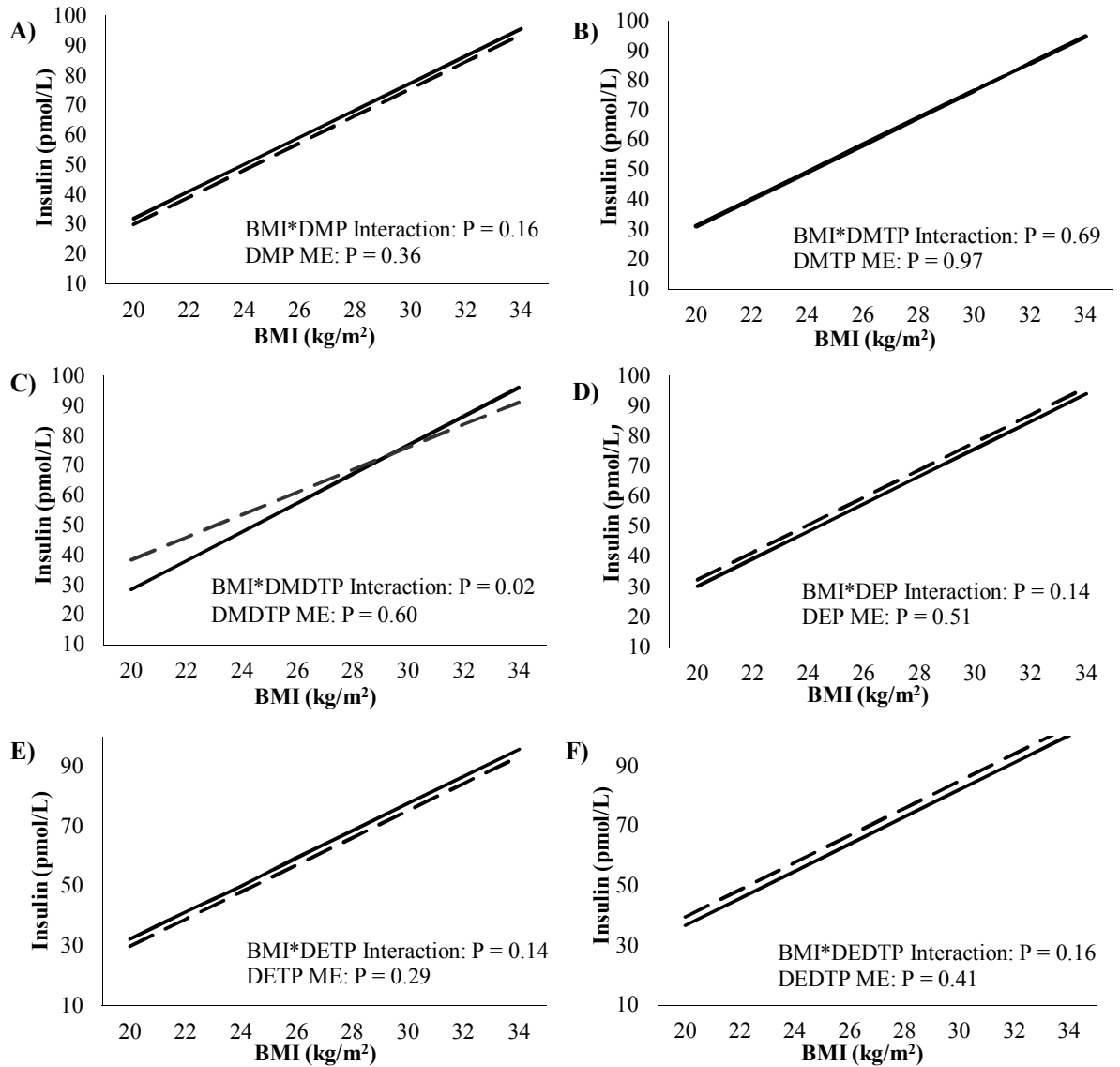


Figure 3 A-F): Relationship between insulin and BMI by DMP (A), DMTP (B), DMDTP (C), DEP (D), DETP (E) and DEDTP (F) detection limit status.

Solid black line represents individuals below the detection limit while the dotted black line represent individuals above the detection limit.

Models are adjusted for PIR, ethnicity, age, sex, smoking status, urinary creatinine, fasting duration and total calorie intake

CHAPTER 4.0 MANUSCRIPT 2

Polycyclic Aromatic Hydrocarbon Biomarkers are Associated with Obesity, Metabolic Syndrome, Dyslipidemia, Hypertension and Type 2 Diabetes

Polycyclic aromatic hydrocarbons (PAH) are man-made environmental pollutants resulting from the incomplete combustion of oil, fossil fuel, gas, coal and waste, and are also produced naturally from volcanoes and forest fires (32). This environmental pollutant normally enters the body through inhalation, but can also gain entry through skin exposure, ingestion of charred/smoked meat, or through the ingestion of contaminated produce and grains cultivated from polluted soil (32). The majority of PAH are excreted from the body shortly after exposure, while small amounts are believed to be retained in fat and liver tissues (32,79). In addition, PAH may accumulate in the body over time (79).

PAHs have been shown to be associated with cancer (80–86), and were recently linked with inflammation (49,50), fatal ischemic heart disease (58), peripheral arterial disease (59) and cardiovascular disease (60). A recent study of PAH in children found a positive correlation between high concentrations of PAH and level of obesity (87). However, it is unknown whether this environmental pollutant is related with obesity in adults and whether the associations between PAH and cardiometabolic conditions remain independent of BMI.

Therefore the objectives of this study are to determine whether PAH is related with obesity and to investigate whether the association between PAH and metabolic

syndrome (MetS), hypertension, diabetes, and dyslipidemia is present, independent of BMI.

RESEARCH METHODS

Participants

Adult participants at least 20 years of age from the National Health and Nutrition Examination Survey (NHANES; 2001-2004 and 2007-2012 survey years) with available PAH data were included in this study. NHANES is a nationally representative survey of the non-institutionalized United States population with microdata available online to the public (63). All individuals provided written informed consent prior to participation.

Participants were excluded from the study if they were pregnant (n=292), had a BMI ≥ 70 kg/m² (n=2) or had missing data for BMI (n=125), poverty income ratio (n=525), smoking status (n=7), or creatinine (n=1). Individuals who could not be categorized into having or not having MetS (n=216) or dyslipidemia (n=124) due to missing data were also excluded resulting in 6159 remaining participants.

Polycyclic Aromatic Hydrocarbons

Trained professionals at mobile examination centers collected urine samples from participants and stored them at -20° C or lower. Capillary gas chromatography as well as high-resolution mass spectrometry was used to quantify the metabolites of interest. A total of 8 hydroxylated urinary PAH metabolites were observed including 1-naphthalene, 2-naphthalene, 2-fluorene, 3-fluorene, 1-phenanthrene, 2-phenanthrene, 3-phenanthrene, and 1-pyrene. After performing regression analyses it was observed that the parameter estimates for the bottom 4 quintiles were very similar, while the top quintile differed

significantly. Therefore, each PAH metabolite was dichotomized into high (highest quintile) and low (lower 4 quintiles).

Metabolic Syndrome, Dyslipidemia, Type 2 Diabetes and Hypertension

Metabolic syndrome (MetS) was defined using the National Heart, Lung, and Blood Institute's updated NCEP ATP III definition (88). Individuals with 3 or more of the following 4 metabolic risk factors were classified as having MetS. These risk factors included: 1) cholesterol abnormalities (HDL ≤ 1.03 mmol/L for men and ≤ 1.29 mmol/L for women, were on cholesterol medication, or had a doctor diagnose them with abnormal cholesterol levels), 2) high triglyceride levels (≥ 1.69 mmol/L), 3) high blood pressure (systolic blood pressure (SBP) of ≥ 130 mm Hg, diastolic blood pressure (DBP) ≥ 85 mm Hg, taking hypertension medication, or had doctor diagnosed hypertension), or 4) blood glucose abnormalities (fasting glucose levels of ≥ 5.6 mmol/L, taking diabetes medication, taking insulin, or had doctor diagnosis diabetes). Waist circumference was excluded as one of the possible risk factors to avoid having obesity as both the dependent and independent variable and allow for the examination of BMI as an independent variable.

Dyslipidemia was defined as: serum triglyceride levels ≥ 2.06 mmol/L, total cholesterol ≥ 6 mmol/L, HDL < 1.04 mmol/L for men and < 1.29 for mmol/L for women, were on cholesterol medication, or had doctor diagnosed hypercholesterolemia. Diabetes was defined as: fasting plasma glucose ≥ 7 mmol/L (89), plasma glycohemoglobin (HbA1c) levels $\geq 6.5\%$ (90), doctor diagnosed T2D, taking diabetes medication, or were

taking insulin. Hypertension was defined as: SBP \geq 140 mmHg, DBP \geq 90 mmHg, doctor diagnosed hypertension, or taking hypertension medication (91).

Statistical Analysis

Differences in participant characteristics by MetS status were examined using chi-square tests for categorical variables and t-tests for continuous variables. The prevalence (N,%) was presented for categorical variables, while the mean \pm standard error (SE) was presented for continuous variables. Multiple linear regressions and partial correlations were performed to study the association between BMI and standardized PAH levels adjusting for age, sex, ethnicity, poverty index ratio (PIR), creatinine (64), and smoking status. It should be noted that due to the high correlation between PAH and smoking (33,34), in addition to other covariates, all multivariable analysis was adjusted for the smoking status variable to account for the possible disadvantageous metabolic outcomes resulting from smoking status.

Adjusted logistic regressions were performed to examine the association between MetS, hypertension, T2D, dyslipidemia and PAH. Each regression model included an interaction term between PAH and BMI, if no significant interaction was observed, the term was removed and the main effects were examined. If a significant interaction was observed, BMI was categorized into normal weight, overweight and obese to further examine the relationship between PAH and metabolic conditions by weight status. Since the prevalence of the outcomes of interest were greater than 10%, the ORs were converted to prevalence risk (PR) using a formula introduced by Zhang et al. (92).

$$PR = \frac{OR}{(1 - P_0) + (P_0 \times OR)}$$

SAS v. 9.4 was utilized for all

statistical analysis, and all models except the Pearson correlations were weighted to be representative of the general US population with significance being observed at $P \leq 0.05$.

RESULTS

Table 1 presents the participant characteristics by MetS status. A total of 1265 (20.5%) individuals were defined as having MetS. In general, those with MetS were significantly older ($P < 0.0001$), had higher BMI levels ($P < 0.0001$) and were more likely to be male ($P = 0.014$).

As can be seen in **Table 2**, BMI was positively associated with 1-naphthalene, 2-naphthalene and 2-phenanthrene ($P < 0.05$) and negatively associated with 2-fluorene, and 3-fluorene ($P < 0.05$) after adjustment for age, sex, ethnicity, PIR, smoking status, and creatinine.

Figure 1A illustrates the association between the various PAH metabolites and MetS after adjusting for confounders. There were no significant interaction effects between PAH and BMI ($P > 0.05$). For a given BMI, individuals in the highest quintile of 1-naphthalene had a 25.5% greater PR of MetS than those with lower levels ($P = 0.0061$). For a given BMI, individuals in the highest quintile of 3-fluorene had 26.9% greater PR of MetS compared to those with lower levels ($P = 0.0143$). No significant associations were observed for the other metabolites and MetS ($P > 0.05$).

No interaction effects were observed for T2D (PAH x BMI interactions, $P > 0.05$). For a given BMI, individuals in the highest quintiles of 1-naphthalene and 2-naphthalene had a 52.5% ($P = 0.0005$) and 42.4% ($P < 0.015$) greater PR of T2D respectively (**Figure 1B**). For a given BMI, those in the highest quintiles of 2-fluorene and 3-fluorene had a 45.4% ($P = 0.0027$) and 46.7% ($P = 0.0035$) greater PR of T2D than

those with lower levels. There were no significant associations observed for the other metabolites and T2D ($P > 0.05$).

Certain PAHs influenced the relationship between BMI and hypertension wherein normal and overweight individuals in the highest quintiles of 2-fluorene and 3-fluorene had a greater PR of hypertension than those with lower levels, while little difference was observed in obese individuals (PAH x BMI interactions: $P = 0.0004$ and $P = 0.017$ respectively, **Figure 2**). Further, for a given BMI, those in the highest quintiles of 1-naphthalene and 2-naphthalene had a 15.3% ($P = 0.0059$) and 15.6% ($P = 0.021$) greater PR of hypertension respectively compared to those with lower levels. No significant associations were observed with 1-pyrene or the phenanthrene metabolites and hypertension ($P > 0.05$).

PAH biomarkers influenced the relationship between BMI and dyslipidemia wherein overweight and obese individuals in the highest quintiles of 1-phenanthrene had a greater PR of dyslipidemia than those with lower levels, while little difference was observed in normal weight individuals (PAH x BMI interaction: $P = 0.0026$, **Figure 3**). For a given BMI, those in the highest quintiles of 2-fluorene, 3-fluorene and 1-naphthalene had a 9.3% ($P = 0.02$), 13.0% ($P = 0.0035$) and 7.2% ($P = 0.046$) greater PR of dyslipidemia than those with lower levels. No significant associations were observed for dyslipidemia and the other metabolites ($P > 0.05$).

DISCUSSION

To our knowledge, we are one of the first to examine the association between PAH and obesity in adults with PAH being observed to be both positively and negatively associated with BMI. In addition, we are one of the first to examine the relationship between PAH and MetS, diabetes hypertension, and dyslipidemia independent of BMI. We observed the naphthalene and fluorene metabolites to have significant deleterious associations with a number of different health risk independent of BMI, while two of the phenanthrene biomarkers and 1-pyrene were not related to any health conditions. These findings bring to light the potentially detrimental impact of certain PAH biomarkers on obesity and metabolic conditions in the general population.

The literature on BMI and PAH relationships has been limited to date (87). Contrary to previous studies conducted on children (87), we observed both a positive and negative association between PAH and BMI. BMI is widely believed to be correlated with higher adipose tissue concentrations. Since PAH is stored in fat tissue (32), it is hypothesized that more PAH will be stored in individuals with higher BMIs leading to lower excretion levels of PAH in urine and feces. This may be a plausible explanation to the negative associations we observed between the metabolites and BMI. Though no causal relationship can be extrapolated here, the weak association between BMI and PAH may be a modest contributing factor to the increasing prevalence of obesity. In addition to the BMI and PAH relationship, we observed PAH to be more strongly associated with the other health risks examined.

MetS is recognized to be related with numerous risk factors including circulatory disease (93), which is of increasing relevance since more than 20% of the US adult population are affected by MetS (94). We observed 2 PAH metabolites to be associated with a greater risk of MetS, independent of BMI. This significant association raises additional questions about the influence of PAH on health, which warrants further investigation.

In addition to MetS, 3 other major risk factors of CVD were examined in this study including T2D. A recent study reports naphthalene biomarkers, and 2-phenanthrene to be associated with T2D independent of BMI (95). Consistent with previous literature, we observed that regardless of BMI, individuals with high levels of naphthalene had a 41-49% greater risk of T2D compared to those with lower levels. However, we did not detect any significant associations between 2-phenanthrene and T2D. In addition, we observed the fluorene biomarkers to augment the risk of T2D, independent of BMI, whereas previous literature (95) failed to demonstrate such a relationship. These differences in findings may in part be due to the different T2D definitions used in the studies, where, in addition to high glycohemoglobin, the current study also included individuals with blood glucose levels ≥ 7 mmol/L. Furthermore, NHANES updated their data regarding PAH which allowed us to examine two additional survey years. In summary, PAH is associated with T2D and may be a factor related to its high prevalence. Considering the pre-established health risks commonly related with T2D (96), these findings warrant the attention of researchers and clinicians caring for T2D patients to monitor PAH levels. In

addition, we still require research to enhance our limited knowledge of the underlying physiological effects of each PAH biomarker on T2D.

To our knowledge, no studies have examined the relationship between hypertension and PAH. We observed that, regardless of BMI, individuals with high levels of 1-naphthalene and 2-naphthalene have a 15% greater PR of hypertension. In addition, high levels of certain PAH biomarkers may be modestly associated with hypertension in normal and overweight individuals but not in the obese. These novel results raise questions about the potential mechanisms of action that allow for greater hypertension risks. A study on rodents suggests that PAH may result in carcinogenesis and atherogenesis by increasing inflammation and the size of plaques in arteries (97). However, the proportion of PAH excreted by obese individuals may be lower than normal weight or overweight individuals because the obese may be storing more of the PAH. Thus, the observations from this study examining urinary metabolites of PAH and not fat storage, could be underestimating the true health effects of PAH in the obese population.

Although studies on potential PAH and hypertension relationships are non-existent, one study was found to investigate the effects of environmental pollutants on lipid markers. In general, the study reports high levels of fluorene biomarkers to be associated with unfavorable HDL levels (56). In the current study, we observed that those with high levels of fluorene had a 7-13% greater PR of dyslipidemia, independent of BMI. These findings are consistent with the previous study as both observed PAH to be related to a disadvantageous lipid profile (56), however, we also observed 1-naphthalene

to be related to dyslipidemia whereas no such findings were demonstrated in previous literature. Furthermore, high levels of 1-phenanthrene augment the risk of dyslipidemia in the overweight and obese but not normal weight individuals. It is important to note that the present study observed PAH to be associated with the condition of dyslipidemia as a whole, which uses cut-offs that have been proven to increase the risk of cardiac-related illnesses. Therefore, this method of measurement may be a better indicator of risk as opposed to isolating each component.

We are aware of a number of potential limitations of our study. Our cross-sectional study design does not allow for extrapolation of causal or temporal relationships between PAH and health risk. In addition, examined urinary concentrations of PAH are reflective of immediate or short-term PAH exposure and do not account for the PAH levels stored in fatty tissue. Therefore we still require studies to investigate PAH levels in fat tissue as it relates to health risk over an extended period of time. Finally, we were unable to study some of the more toxic and carcinogenic PAHs including benzopyrene (32,35), as NHANES failed to collect such data. However, an important strength of this study is the fact that we are one of the first to examine the association between metabolic conditions and PAH, independent of BMI. In addition, all multivariable statistics models were adjusted for smoking status, which allowed us to account for the underlying variability caused by cigarette smoke inhalation. Furthermore, these results are representative and generalizable to the general adult population.

In conclusion, PAH is associated with greater risk of cardiometabolic health conditions independent of BMI. Those with high levels of PAH have a greater risk of

MetS, hypertension, dyslipidemia and T2D. This suggests future studies should explore the physiological mechanisms of each biomarker on health.

Table 1: Participant characteristic and metabolic syndrome status

	No MetS (n = 4894)	MetS (n = 1265)	<i>P</i>
Age (years)	43.8 ± 0.3	56.3 ± 0.5	<0.0001
BMI (kg/m ²)	27.6 ± 0.1	32.0 ± 0.3	<0.0001
Creatinine (mg/dL)	125.7 ± 1.6	120.2 ± 2.6	0.0350
Sex, <i>n</i> (% male)	2446 (50.0)	683 (54.0)	0.0140
Ethnicity, <i>n</i> (%)			<0.0001
White	2317 (47.3)	639 (50.5)	
Black	1074 (21.9)	271 (21.4)	
Other	1503 (30.7)	355 (28.1)	
Smoker, <i>n</i> (%)			0.0104
Never	2642 (54.0)	601 (47.5)	
Current	1117 (22.8)	264 (20.9)	
Past	1135 (23.2)	400 (31.6)	
PIR, <i>n</i> (% below poverty)	916 (18.7)	243 (19.2)	0.9585
Hypertension, <i>n</i> (%)	1444 (29.5)	991 (78.3)	<0.0001
Type 2 Diabetes, <i>n</i> (%)	303 (6.2)	594 (47.0)	<0.0001
Dyslipidemia <i>n</i> , (%)	2545 (52.0)	1238 (97.9)	<0.0001
<i>PAH metabolites</i> (ng/L)			
1-naphthalene	28935 ± 9935	29478 ± 4550	0.9755
2-naphthalene	7658 ± 278	7627 ± 398	0.9351
2-fluorene	711 ± 27	751 ± 52	0.3449
3-fluorene	359 ± 15	366 ± 29	0.7769
1-phenanthrene	238 ± 8	251 ± 17	0.3720
2-phenanthrene	96 ± 4	104 ± 9	0.3019
3-phenanthrene	192 ± 10	189 ± 18	0.8993
1-pyrene	182 ± 9	174 ± 15	0.5496

Abbreviations: MetS, metabolic syndrome; BMI, body mass index; PIR, poverty index ratio

Continuous variables are presented as mean ± SE

Table 2: The association between PAH and BMI

PAH metabolite	Regression Parameter Estimate		Partial Pearson r	
	$\beta \pm \text{SE}$	<i>P</i>	<i>r</i>	<i>P</i>
1-naphthalene	0.22 \pm 0.1	<0.01	0.028	0.03
2-naphthalene	0.26 \pm 0.1	0.04	0.002	0.90
2-fluorene	-0.04 \pm 0.1	0.79	-0.043	<0.01
3-fluorene	-0.43 \pm 0.2	0.05	-0.082	<0.01
1-phenanthrene	0.13 \pm 0.1	0.21	0.017	0.19
2-phenanthrene	0.38 \pm 0.1	<0.01	0.051	<0.01
3-phenanthrene	-0.10 \pm 0.1	0.31	-0.008	0.52
1-pyrene	-0.02 \pm 0.1	0.79	-0.016	0.20

PAH is standardized

All regression models were weighted

All regression and partial Pearson *r* values are adjusted for age, sex, ethnicity, PIR, smoking status and creatinine



Figure 1: The prevalent ratio (PR) of metabolic syndrome (MetS) (A) and type 2 diabetes (T2D) (B) for those in the highest quintiles of PAH compared to lower levels

* = Significant PR at $P < 0.05$

Weighted and adjusted for age, sex, BMI, ethnicity, poverty index ratio, smoking status, and creatinine

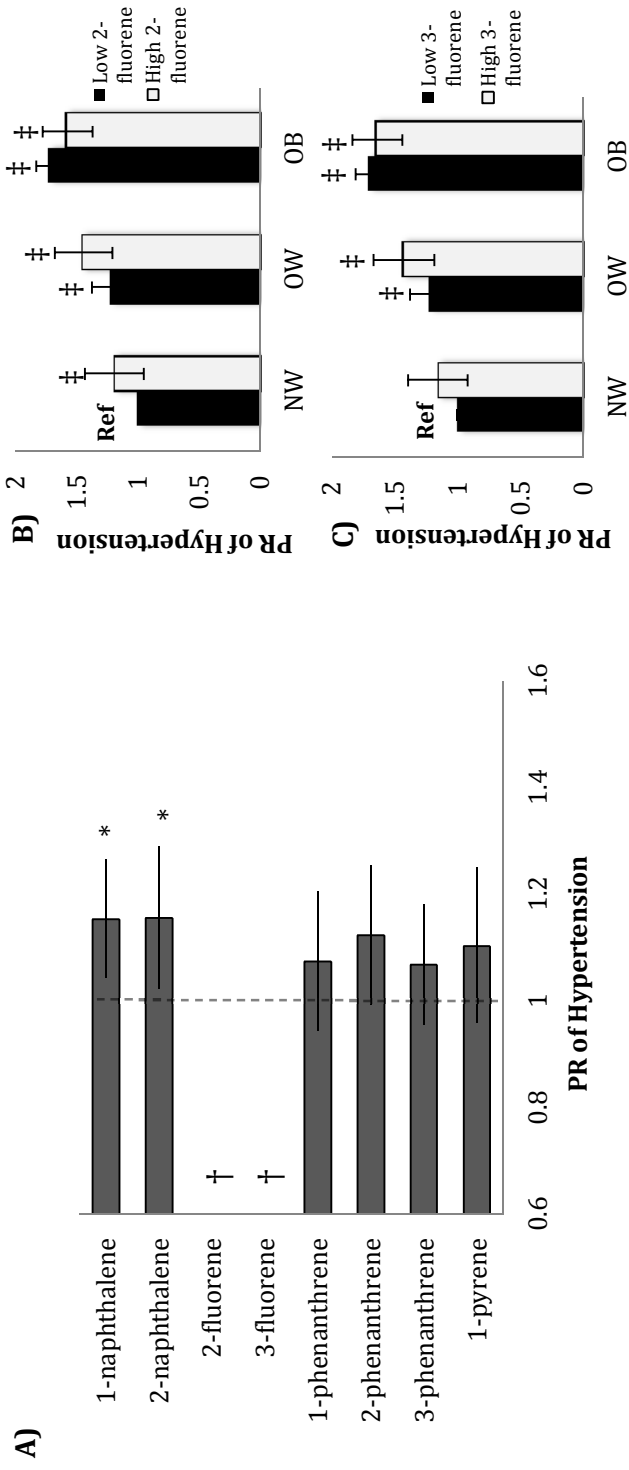


Figure 2: Prevalent ratio (PR) of hypertension for those in the highest quintiles of PAH compared to lower levels

Panel A is adjusted for age, sex, BMI, ethnicity, poverty index ratio, smoking status, and creatinine

Panel B and C are stratified by weight status to show the BMI – PAH interaction effect for 2-fluorene (B) and 3-fluorene (C) and adjusted for age, sex, ethnicity, poverty index ratio, smoking status, and creatinine

White bars are the highest quintile and black bars are the lower quintiles

* = Significant PR ($P < 0.05$)

† = Significant interaction effect between PAH and BMI

‡ = Significant ($P < 0.05$) difference between the highest quintile of 2-fluorene compared to lower levels

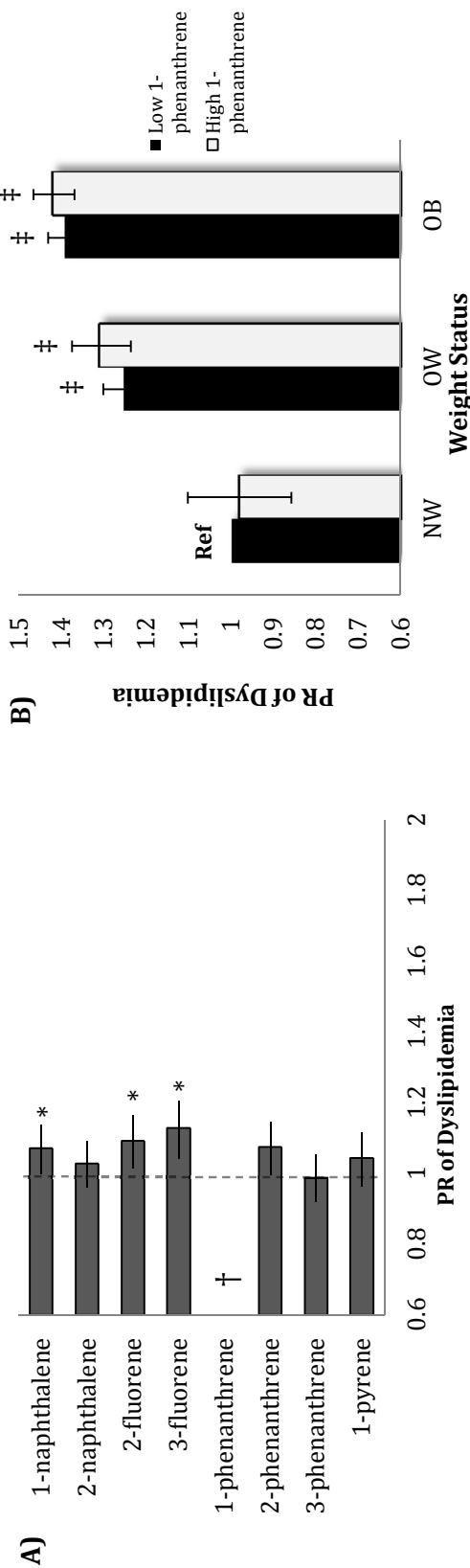


Figure 3: Prevalent ratio (PR) of dyslipidemia for those in the highest quintiles of PAH compared to lower levels

Panel A is adjusted for age, sex, BMI, ethnicity, poverty index ratio, smoking status, and creatinine

Panel B is stratified by weight status to show the BMI – PAH interaction effect for 1-phenanthrene and adjusted for age, sex, ethnicity, poverty index ratio, smoking status, and creatinine

White bars are the highest quintile and black bars are those in the lower quintiles

* = Significant PR ($P < 0.05$)

† = Significant interaction effect between PAH and BMI

‡ = Significant ($P < 0.05$) difference between the highest quintile of 1-phenanthrene compared to lower levels

CHAPTER 5.0 GENERAL DISCUSSION

The overall health of an individual is dependent on a number of different components including physical, psychological, and environmental. There has been a rise in cardiometabolic conditions including obesity, T2D, dyslipidemia and hypertension that are augmenting the risk of CVD and mortality in the general population. With the growing prevalence and the subsequent economic burden related to these conditions, researchers have been compelled to explore the myriad of diverse risk factors related to health. Prior to this thesis, it was unclear whether PAH or the less studied OP pesticides were associated with cardiometabolic risk factors, independent of BMI, however, the studies in this thesis have attempted to address the gaps in the literature pertaining to these exposures.

Based on the first manuscript, we can conclude that various types of OP can have either detrimental or advantageous associations with health for a given BMI in the general population. However, based on these results, it is important to note that each metabolite may relate differently to health risk. We recommend future researchers to focus on the mechanisms that may explain these observations.

The relation between PAH and cardiac health has been previously established. However, research is lacking in regards to the relationship between PAH and risk factors of CVD. In the second manuscript, we observed PAH to be correlated negatively and positively with obesity. Further, PAH is associated with an increased risk of MetS, hypertension, T2D and dyslipidemia, independent of BMI, and may alter how BMI is

related to health. These novel findings further our understanding of PAH on health and expand the limited body of literature pertaining to this environmental pollutant.

A couple of important limitations must be addressed. First, both studies were cross-sectional in nature so we are unable to infer causation. Second, urinary OP and PAH levels were measured through one spot-urine sample. A more precise measurement indicative of average exposure may have been obtained if there were spot-urine samples acquired on a number of different days, however NHANES is not a longitudinal study and hence did not provide follow-up data collection. A great strength of this study is the use of tandem mass chromatography/mass spectrometry method of quantifying urinary OP exposure levels which has been demonstrated to be a highly sensitive and selective (61,98–100) method of measurement. This is especially important when measuring population levels of OP exposure. In addition, PAH was measured using a combination of solid-phase extraction, capillary gas chromatography and high-resolution mass spectrometry which have also been established to result in highly reliable measurements (101–103).

These studies help grow the modest body of literature pertaining to OP, PAH, obesity and health. Based on these findings, it is clear that certain chemicals the general population comes into contact with may be risk factors for disease. These risk factors may help explain the recent rise in cardiometabolic conditions. However, the environmental exposures examined in this thesis are only a small fraction of the chemicals that individuals come into contact with on a daily basis, and their biological impact is not fully understood. Therefore, it is hoped that the studies in this manuscript

raise the interest of researchers in the environmental science and cardiometabolic health fields to help provide further insight on the impact of environmental pollutants on health and the mechanistic pathways involved.

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